Single cell RNA sequencing data analysis
2026

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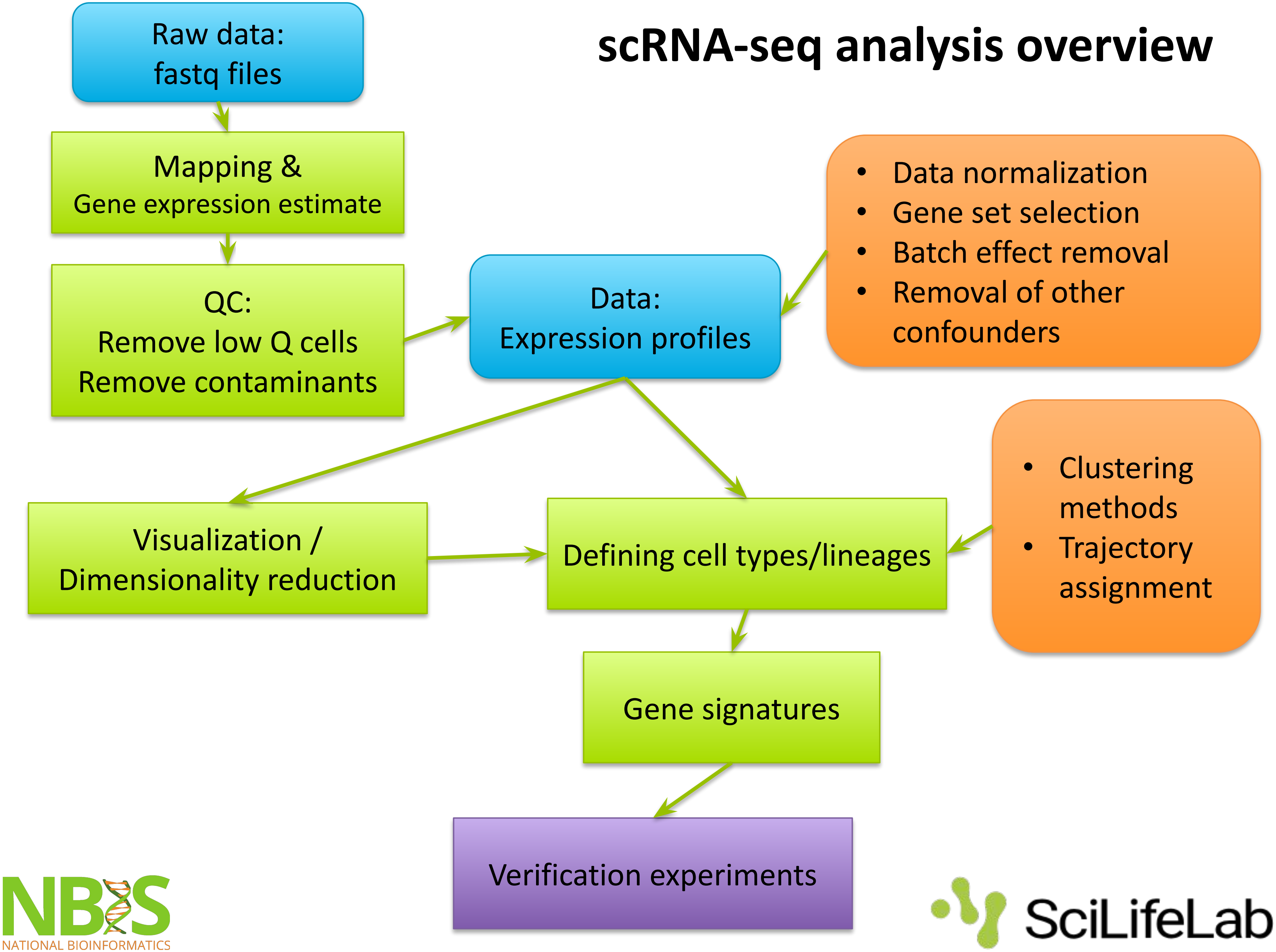
scRNA-seq analysis overview



Raw data:
fastq files

Mapping &
Gene expression estimate

QC:
Remove low Q cells
Remove contaminants

Data:
Expression profiles

- Data normalization
- Gene set selection
- Batch effect removal
- Removal of other confounders

Visualization /
Dimensionality reduction

Defining cell types/lineages

- Clustering methods
- Trajectory assignment

Gene signatures

Verification experiments

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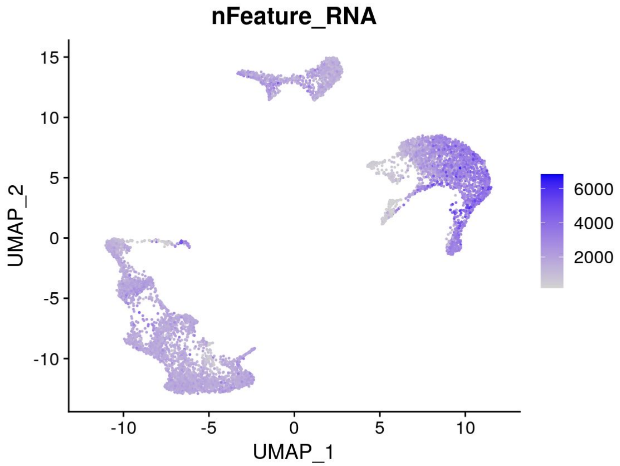
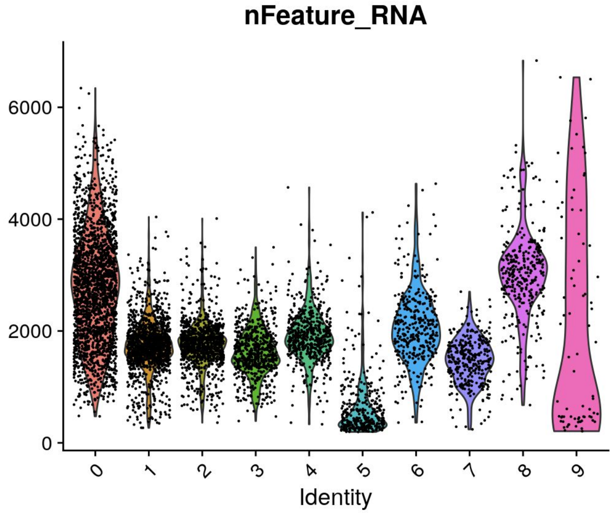
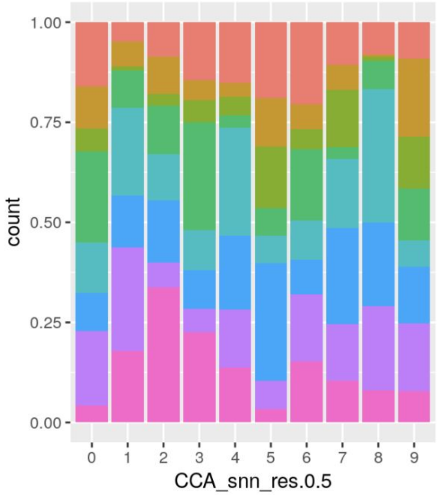
- Data analysis is very seldom a straight line – one pipeline fits all.
  - Often requires several iterations of filtering data, exploring data, refiltering, exploring again, discovering technical artifacts, normalization, exploring again, etc. etc.

- Get to know your data – what types of variation do you have?
  - PCA/UMAP is a good tool for exploring data
- Apply appropriate methods to control for problems that you see.

- Always check for:
  - Batch effects – think of all possible batches.
  - Cell cycle effects if appropriate
  - Separation due to nUMI / nGene / percent mito
- Both at the start of a project and at the end for your final clustering.







- Variable gene selection is a very critical step
  - Filter too much and you may lose populations
  - Keep too much and you may have too much noise

- Similar for choice of PCs

- Clustering – try out a few different approaches
  - Consensus of different methods gives confidence
  - If they do not agree – figure out why!

- Use your biological knowledge to evaluate the results
- Warning! Do not overfit your data to fit your initial hypotheses. Keep an open mind ;-)

- Remember that bioinformatics tools are giving predictions not the truth – always keep a critical mind!
  - Clustering
  - Differential expression
  - Gene set analysis
  - Cell type prediction

- In this course we point out many of the problems that can occur..
- Do not worry too much, in most cases, a standard workflow works well!

- scRNAseq analysis is a fast evolving field with new methods being published all the time.
  - Try to keep up with development
  - **BUT!** You cannot test every new method out there!

Reproducible research in R

- R / Rstudio in Docker containers
  - https://www.andrewheiss.com/blog/2017/04/27/super-basic-practical-guide-to-docker-and-rstudio/
  - https://github.com/rocker-org/rocker
- OBS! On Uppmax/PDC – only Singularity containers are allowed. Most Docker images can be converted.
- Learn more on containers etc:
  - http://nbis-reproducible-research.readthedocs.io/en/latest/
- Rstudio package management – Renv
  - https://rstudio.github.io/renv
- Conda installations of packages – can use conda on both bianca and rackham – `module load conda`

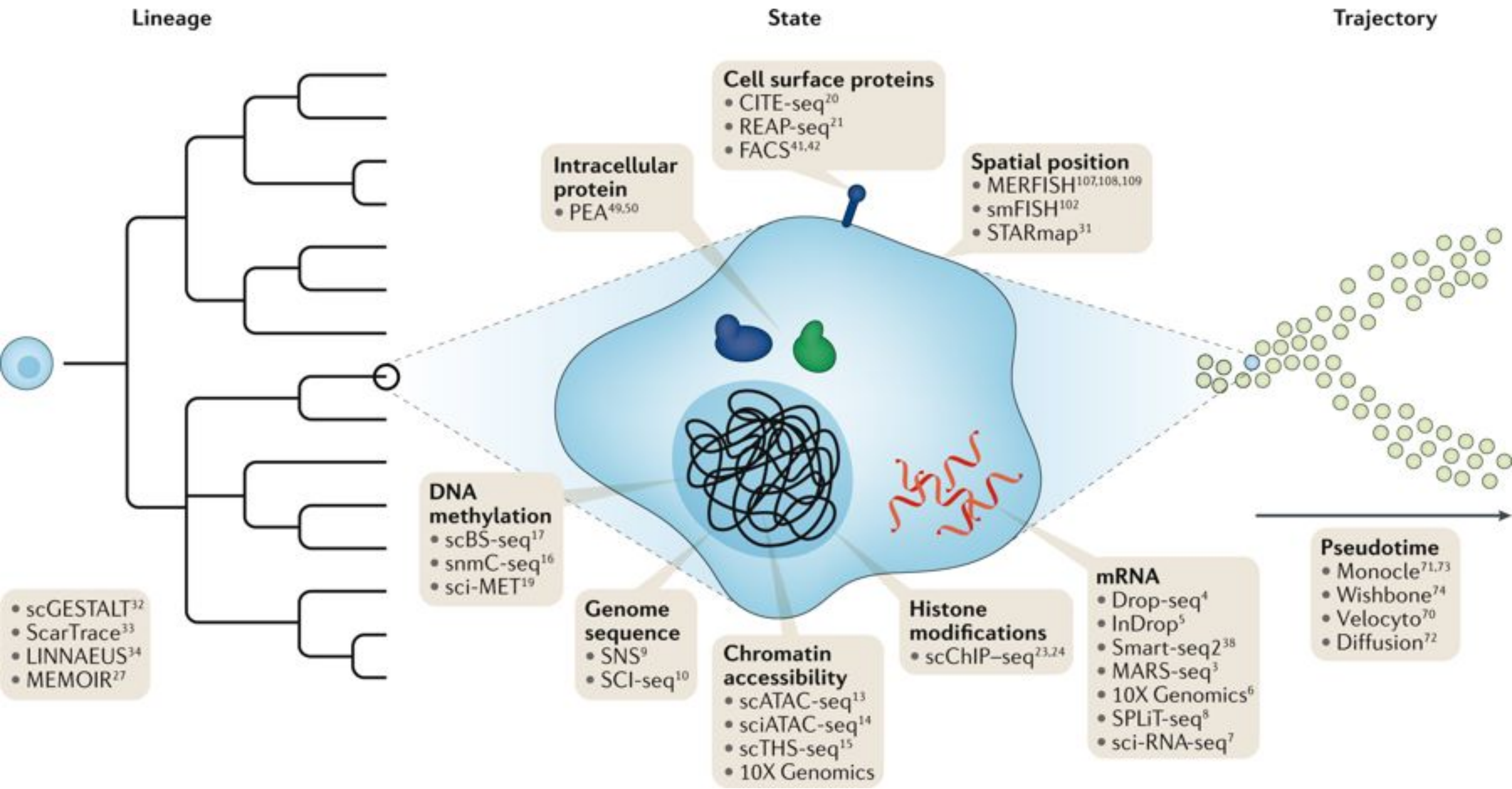
NBIS course in reproducible research:
https://nbisweden.github.io/workshop-reproducible-research/

Compute resources

- In these exercises the datasets were small, but you may have many more cells/samples.
- Structure your code to avoid duplication of matrices and expansion of sparse matrices
  - `rm() & gc()`
- Plan ahead for compute resources, local computer, uppmax or other HPC clusters.
- Human data – raw reads only on encrypted servers like Bianca. Anonymized count matrices are usually fine to analyze in other places.

- We have covered the basic processing, but there is much more you can do…

Single cell omics



Lineage

State

Trajectory

- scGESTALT[32]
- ScarTrace[33]
- LINNAEUS[34]
- MEMOIR[27]

Cell surface proteins
- CITE-seq[20]
- REAP-seq[21]
- FACS[41,42]

Intracellular protein
- PEA[49,50]

Spatial position
- MERFISH[107,108,109]
- smFISH[102]
- STARmap[31]

DNA methylation
- scBS-seq[17]
- snmC-seq[16]
- sci-MET[19]

Genome sequence
- SNS[9]
- SCI-seq[10]

Chromatin accessibility
- scATAC-seq[13]
- sciATAC-seq[14]
- scTHS-seq[15]
- 10X Genomics

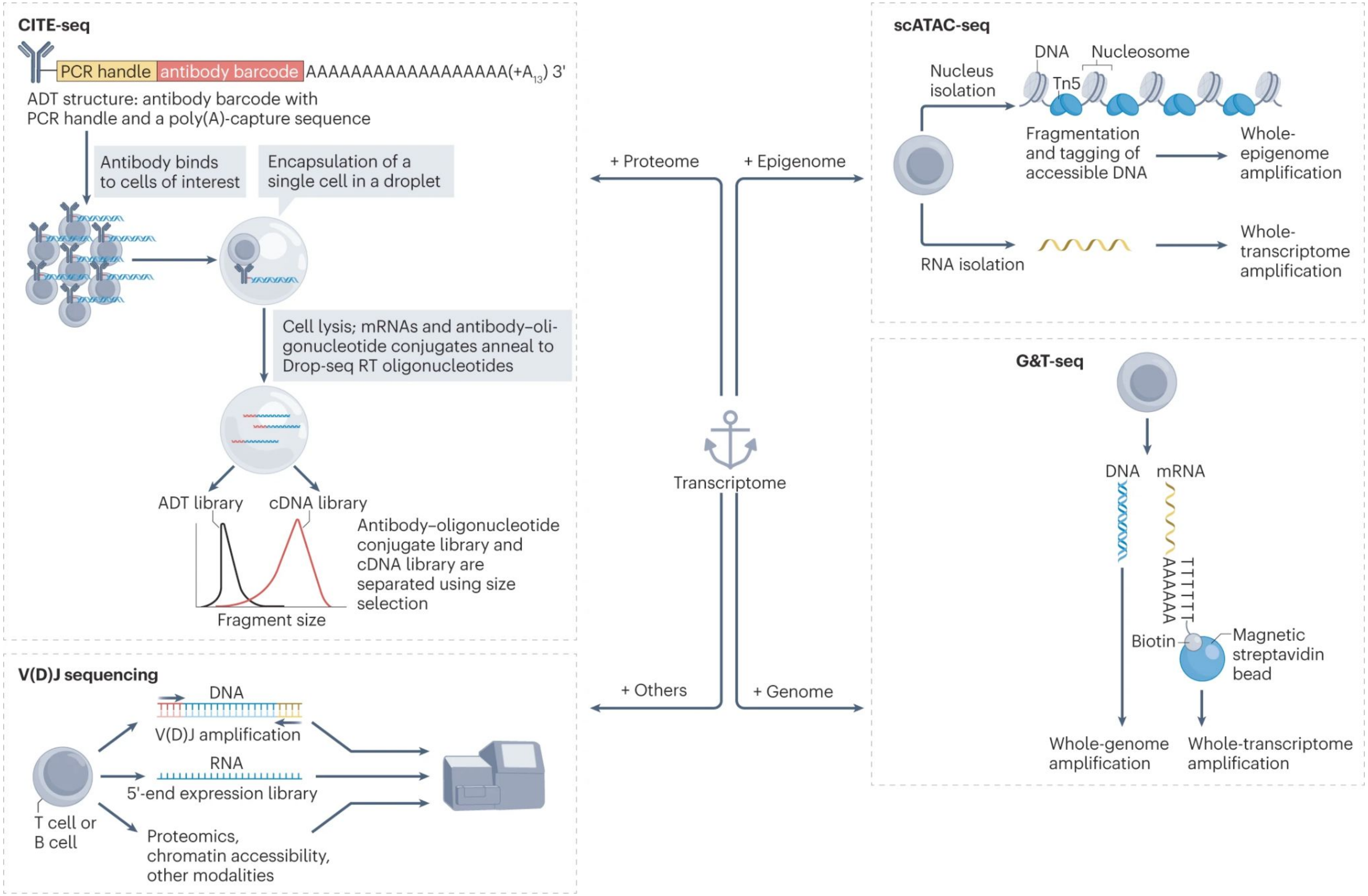
Histone modifications
- scChIP–seq[23,24]

mRNA
- Drop-seq[4]
- InDrop[5]
- Smart-seq2[38]
- MARS-seq[3]
- 10X Genomics[6]
- SPLiT-seq[8]
- sci-RNA-seq[7]

Pseudotime
- Monocle[71,73]
- Wishbone[74]
- Velocyto[70]
- Diffusion[72]

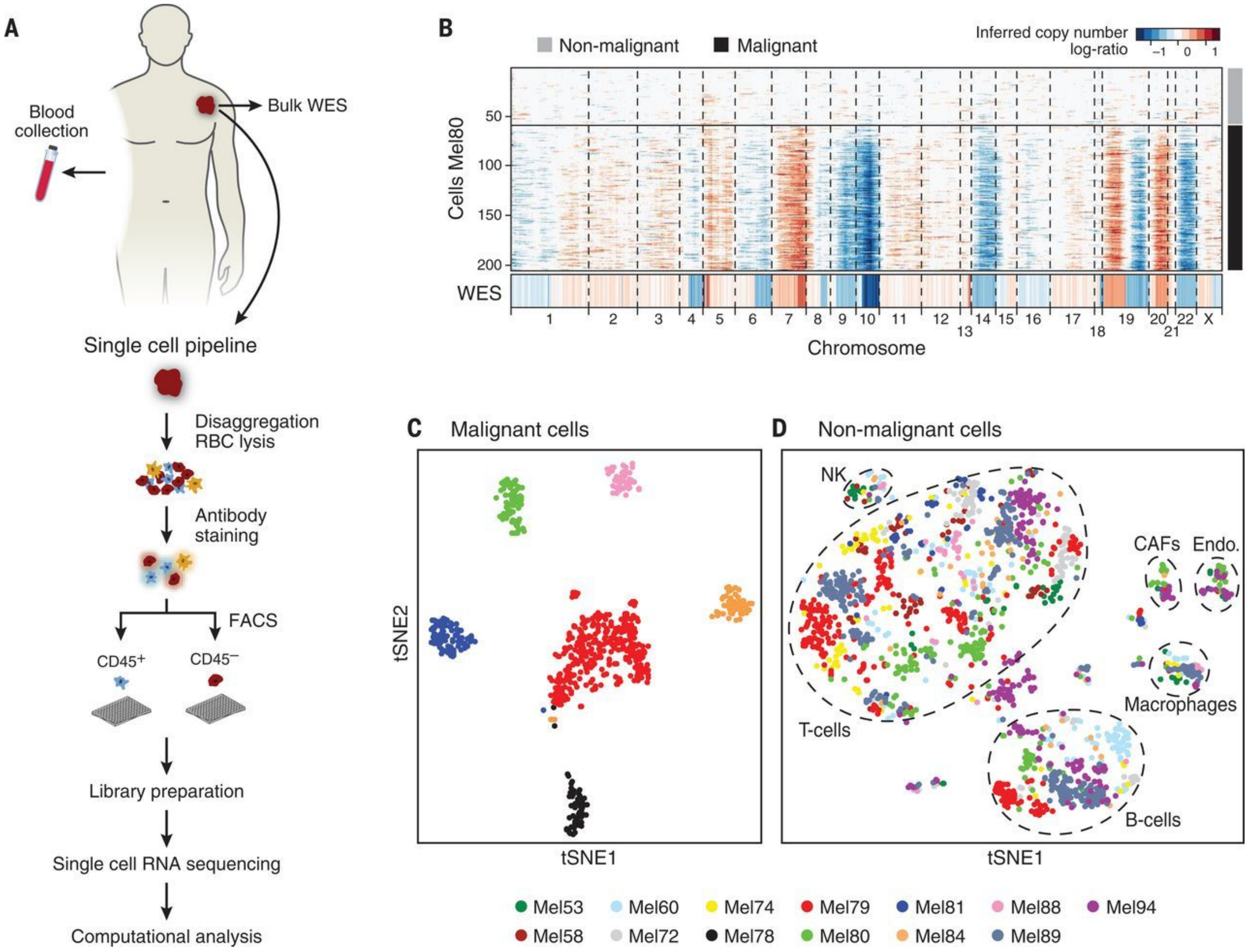
(Stuart & Satija, Nature Rev. Genetics 2019)

SC Multimodal omics



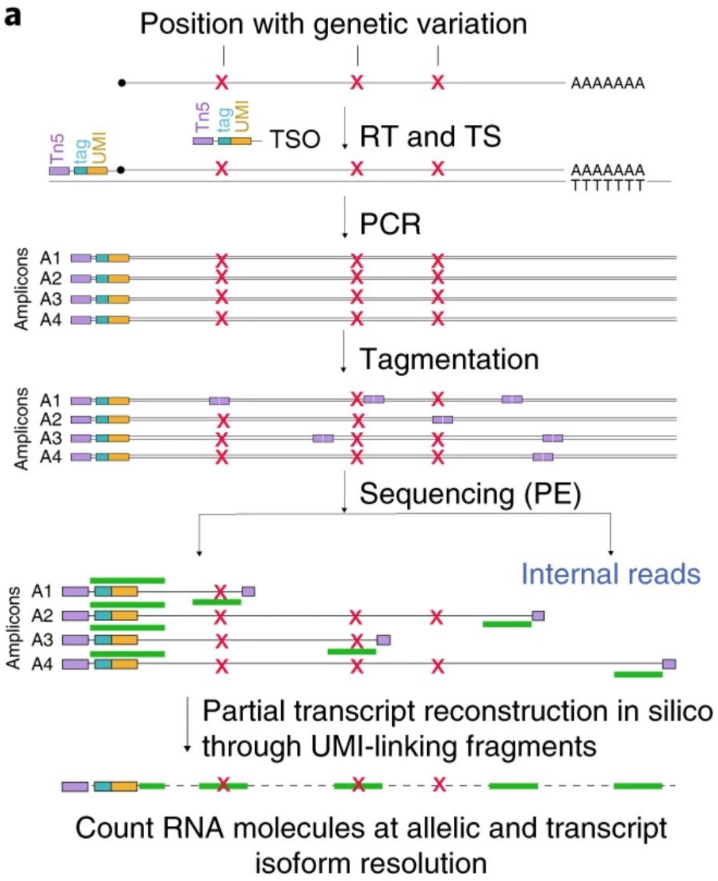
(Baysoy et al, Nat Rev Mol Cell Biol, 2023)

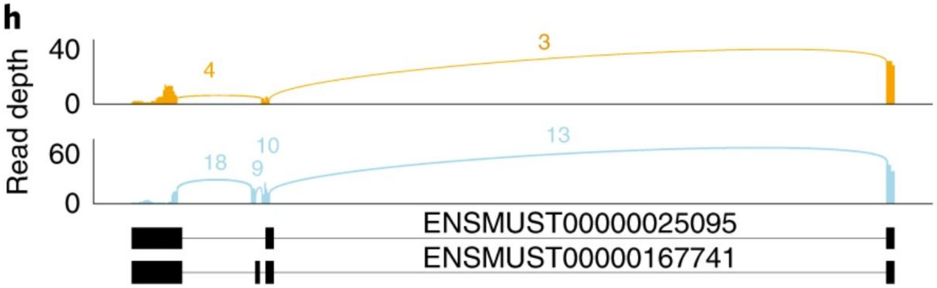
Copy-number variation (CNV) profiling with RNAseq



(Tirosh et al. *Science* 2016)

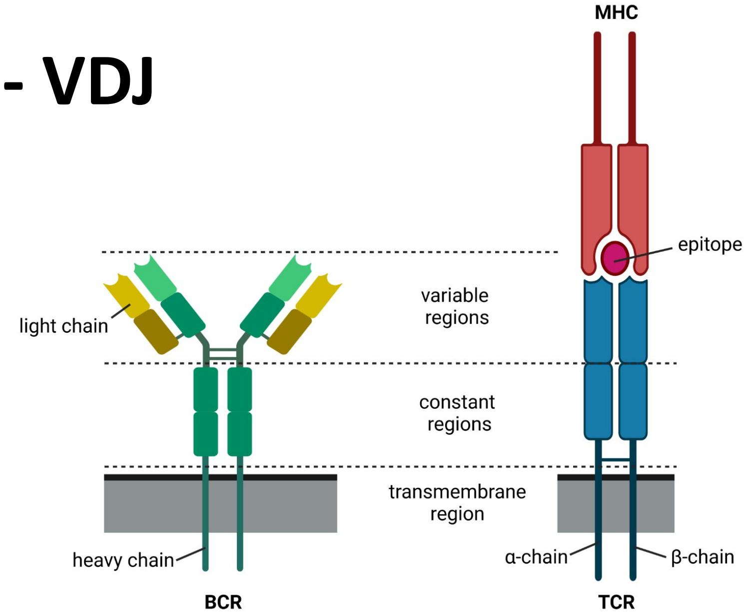
Allele and isoform information with SmartSeq3

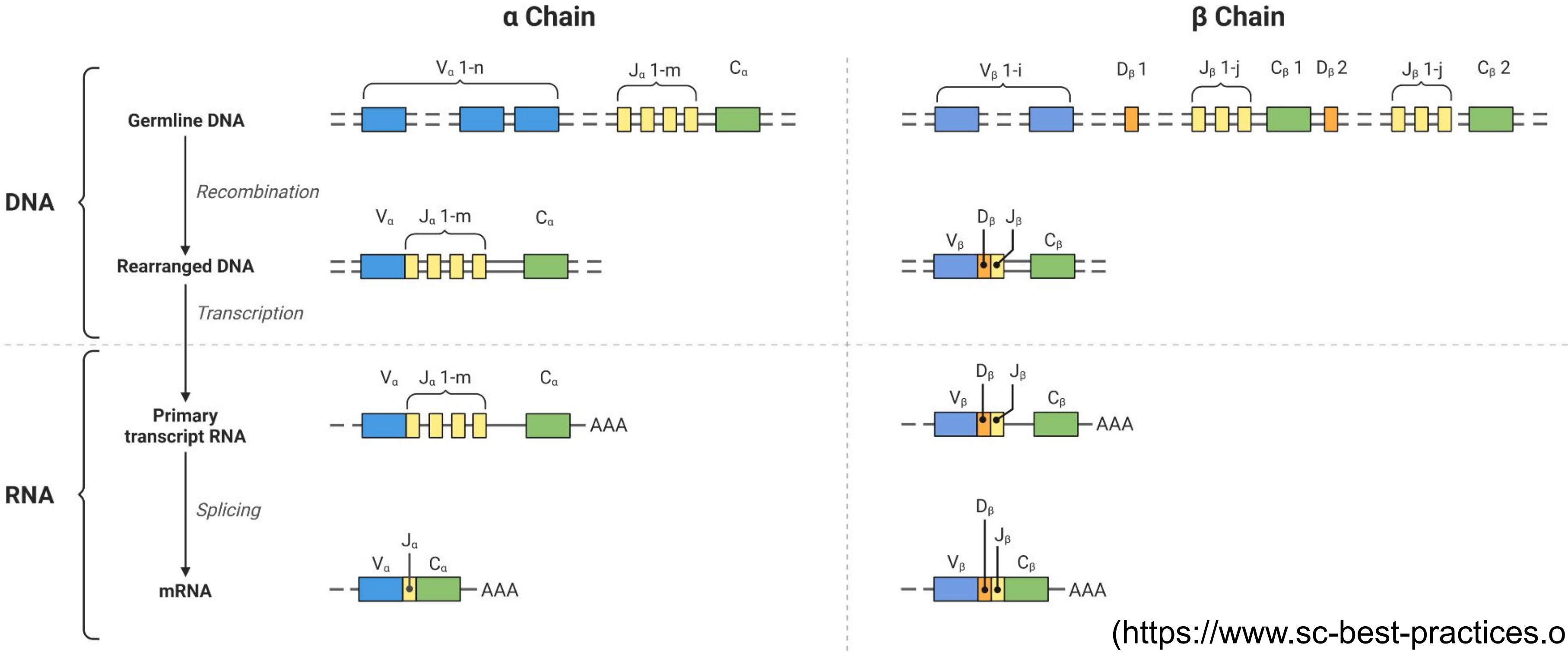




(Hagemann-Jensen et al. Nat. Biotech 2020)

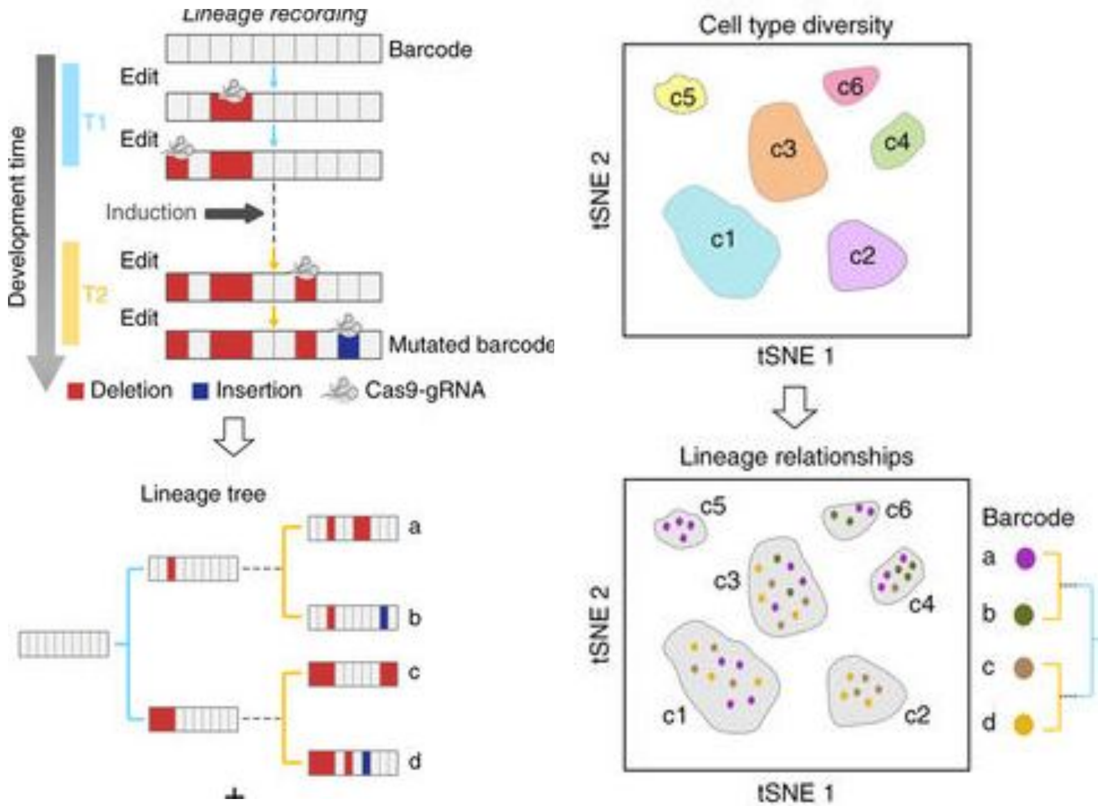
Immune receptor repertoire - VDJ

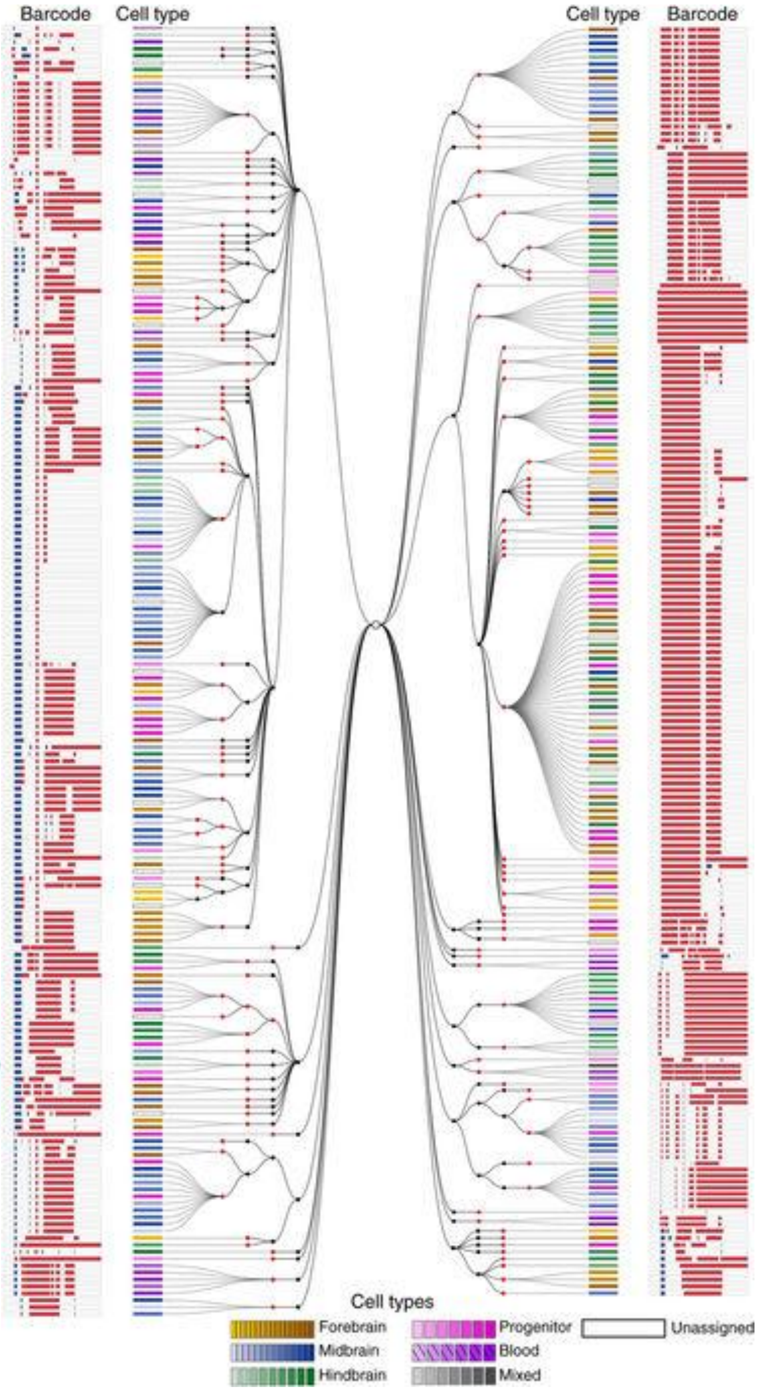




(https://www.sc-best-practices.org)

scGESTALT – lineage tracing and cell profiling with CRISPR-Cas9 editing of barcodes





(Raj et al. Nature Biotech 2018)

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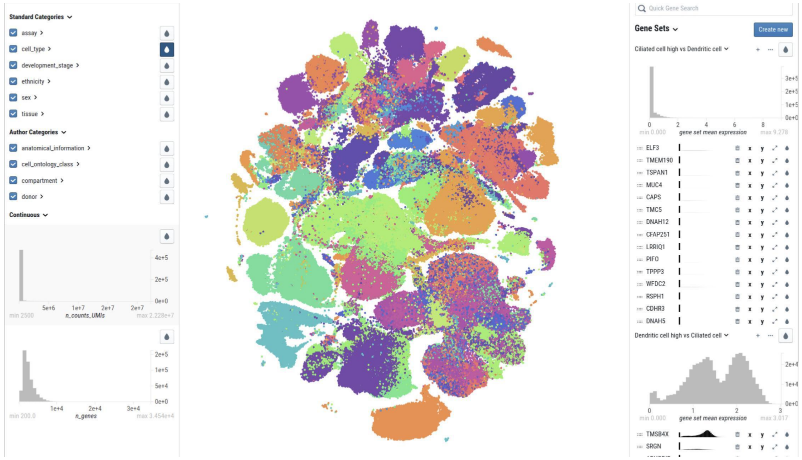
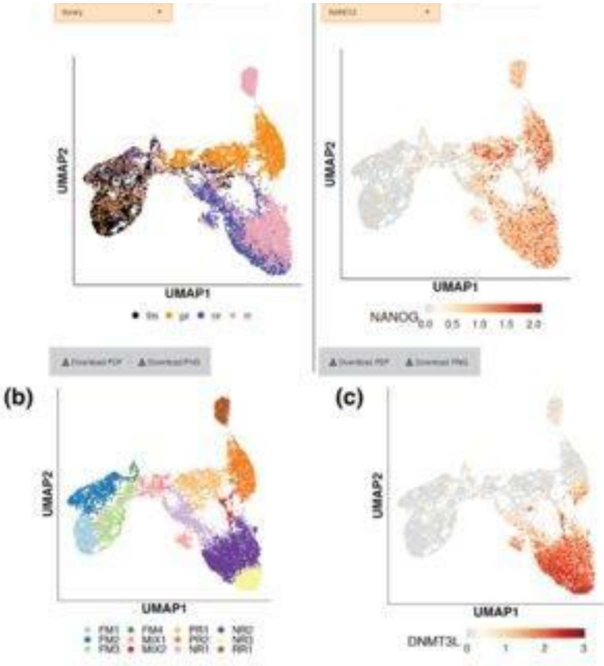
Interactive visualization

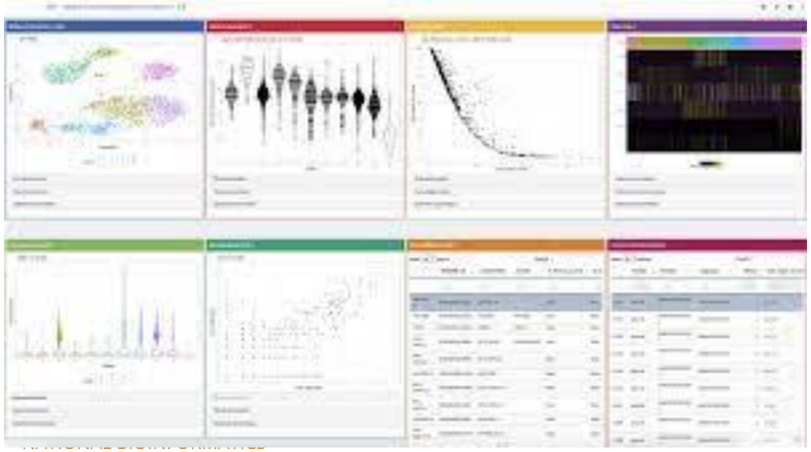
Cellxgene



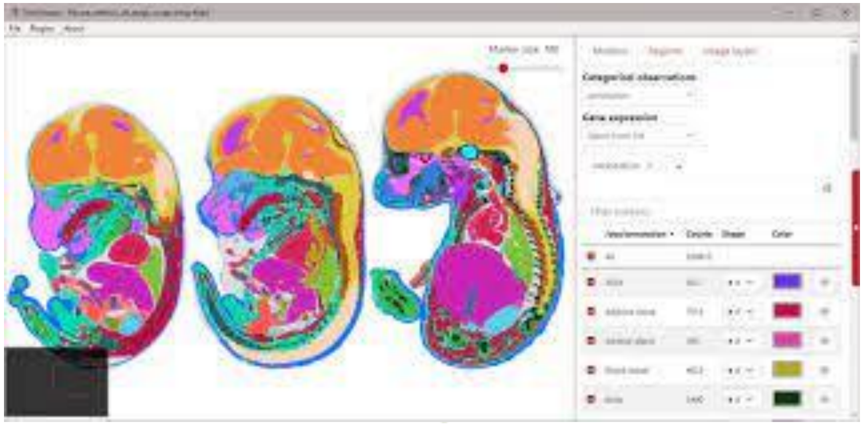
Shinycell



iSEE



TissUUmaps



Some resources

- Course at: https://hemberg-lab.github.io/scRNA.seq.course/
- Scanpy course: https://www.sc-best-practices.org/
- Orchestrating Single-Cell Analysis with Bioconductor http://bioconductor.org/books/3.13/OSCA/
- Many of the packages have good tutorials on their websites
- Repo with scRNA-seq tools: https://github.com/seandavi/awesome-single-cell

Need help?

- NBIS project support
- Courses in programming and other types of analyses.
- Drop-in sessions every Tuesday 14.00
- More info at: http://nbis.se/

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Please fill in the Evaluation Form

Your feedback is important so that we can help improve the course.

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Bring Your Own Data

BYOD April 27th

9:00 - Short introduction

9:15 - Data managers present

You work on your own and we will be around to help.

14:30 - Presentations of all your projects.

16:00 - Summary of the day.

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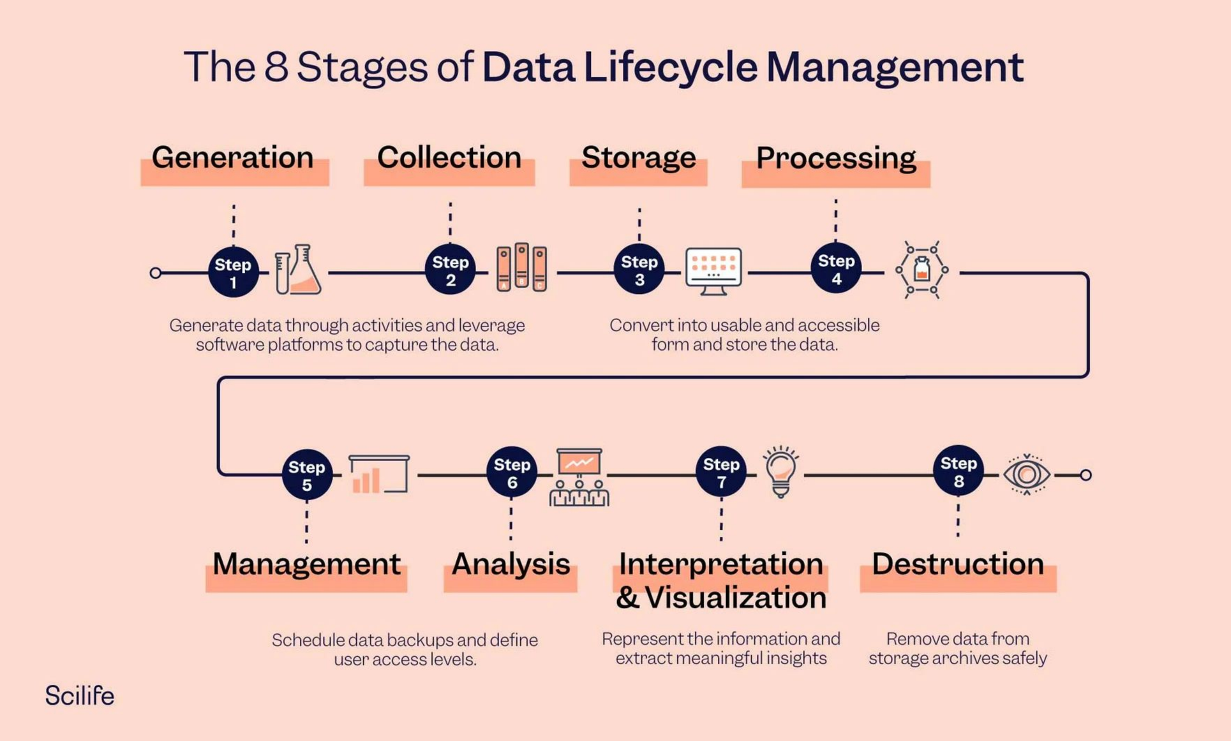
Data Management



The 8 Stages of **Data Lifecycle Management**

Generation — Step 1

Collection — Step 2

Generate data through activities and leverage software platforms to capture the data.

Storage — Step 3

Processing — Step 4

Convert into usable and accessible form and store the data.

Management — Step 5

Analysis — Step 6

Schedule data backups and define user access levels.

Interpretation & Visualization — Step 7

Represent the information and extract meaningful insights

Destruction — Step 8

Remove data from storage archives safely

Scilife

NBIS Data Managers - our guides through the data life cycle will have a short presentation and be present for consultations during the day.

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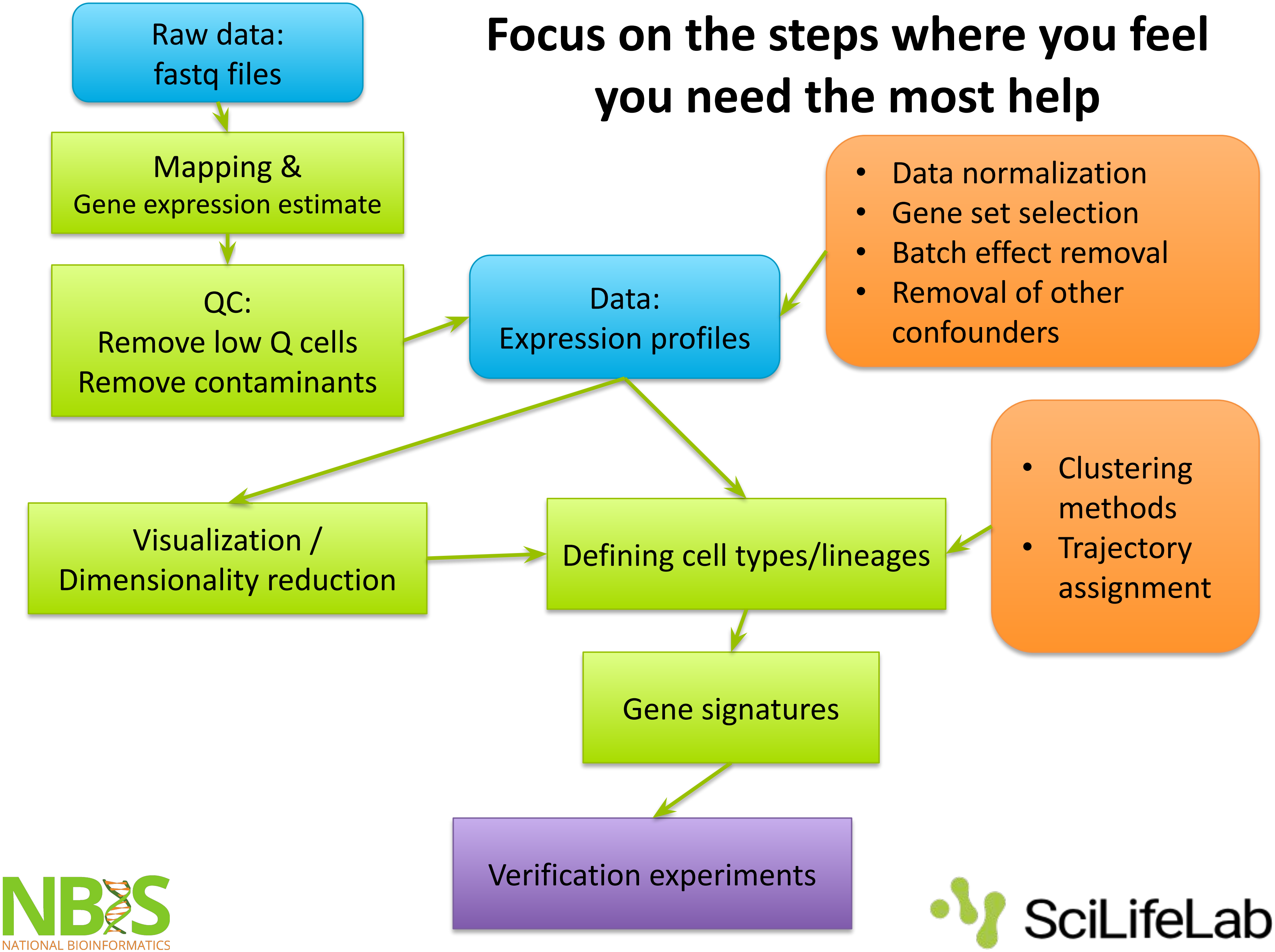
Datasets

- If you have your own data - use that!
- If not - select a dataset from a tissue/celltype that you are interested in.
- Do not select a very large dataset, will be too much computational time during one day!
  - Subsample the cells or select another dataset.
- **If you have sensitive human data, make sure you have permission to work on count matrices locally or on the server.**

Focus on the steps where you feel you need the most help



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Breakout rooms

Please answer our short survey (link also in slack)

https://docs.google.com/forms/d/e/1FAIpQLSeAr90_n7VmgxhZDq5fIhY_XW6CbSoFcrOddNMaRh0UEu3eWw/viewform

We will try to make groups that make sense.

If you know that you won’t attend: Please fill in the survey to let us know!

Compute environments

- If you have a small dataset - less than 50K cells, probably okay to work on the Serve system. Upload it using the Serve File Manager
- For large datasets, use one of the following options:
  - Downsample the dataset and upload to Serve
  - Use the course containers and Docker locally
  - Use a local setup (at your own risk)
    - Conda-files are available on GitHub but not tested outside of the containers
    - Packages can be installed as you go, but different versions can cause issues with dependencies
    - If you haven't already - consider reproducibility! https://uppsala.instructure.com/courses/107067
- Make sure it runs before April 27th
- Ask in the slack channel **#precourse** if you have problems.

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More information

https://nbisweden.github.io/workshop-scRNAseq/other/containers.html

- Option A - running labs on Serve.
  - Use the File Manager at the bottom of the project dashboard to upload your data.
- Option B - running labs locally.

Presentation

We want a **very** short - max 5 minutes presentation of what you have done.

- 1 slide
- What dataset
- Which analysis steps did you perform?
- Which were your main results?
- What were the biggest problems you encountered?

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QUESTIONS?

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